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HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON OVIDUCT OF LAYING AND NON-LAYING EMU BIRDS (*DROMAIUS NOVAEHOLLANDIAE*)

K. VIJAYAKUMAR[1], S. PARAMASIVAN[2] & N. MADHU[3]

[1,3]*Postgraduate scholar, Department of Veterinary Anatomy and Histology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu. India*

[2]*Associate Professor, Department of Veterinary Anatomy and Histology, College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu. India*

ABSTRACT

The microanatomical observation was carried out on oviducts of 12 mature female emu birds at their non-laying (n=6) and laying (n=6) stage. The funnel shaped part of the infundibulum was devoid of mucosal folds. In the tubular part the folds were relatively well developed and showed secondary and tertiary folds lined by pseudostratified ciliated columnar epithelium with goblet cells. The magnum showed tall and broad mucosal folds with secondary folding. The secondary mucosal folds evidenced the epithelial invaginations into the lamina propria of laying birds. The tubular glands were more in number and closely packed together in all mucosal folds of the magnum in laying emu birds.The secondary folds were seen branching from the primary mucosal folds at various heights. The wall of the shell gland was thicker than that of the magnum and the isthmus. The mucosal folds in the shell gland were numerous numbering more than 50 in laying emu birds.. The shell gland region was highly vascular with large arteries and veins observed between the muscle layers. A secondary plexus of smaller arteries and veins were observed in the lamina propria at the base of the mucosal folds. The mucosa of the vagina was composed of pseudostratified ciliated columnar epithelium. The lining of the vaginal folds was a combination of ciliated, non ciliated, vesicular cells. The goblet cells in the epithelium of mucosal folds reacted strongly for PAS staining. The microanatomy of oviduct of emu bird was compared to that of other avian species.

KEYWORDS: Emu, Uterus, Oviduct, Hitology, Histochemistry

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Original Article

INTRODUCTION

The emu is one of the most important wild birds providing oil, leather, feather and meat to the human population. Four distinct types of emus occurred in native Australia before European settlement; only the main land form survives *(Dromaius novaehollandiae).* In birds, normally the left ovary is functional and only the left oviduct is well developed as the right one being vestigial except in raptors. Atretic follicles are prominent feature of the avian ovary and numerous follicles undergo preovulatory atresia at different stages of maturity. Emus usually begin to reproduce between 2 and 3 years of age. However, some birds lay as early as 18 months. Average egg production is about 20-50 eggs per year.

The avian reproductive system has been studied extensively in some poultry birds, specially the domestic fowl and commercial layer birds. The study on the reproductive system of wild birds is necessary to improve their production and reproduction efficacy and to preserve their species. Though preliminary work had been done on the

gross and microscopic anatomical aspects of the reproductive organs in male and female emu, focus detailed histological features of oviduct in laying and non-laying birds needs further study. Hence, an attempt has been made to study the histology and histochemistry of the oviduct during laying and non-laying periods in emu birds.

MATERIALS AND METHODS

The present study on emu bird was conducted at the Department of Veterinary Anatomy and Histology, Veterinary College and Research Institute, Namakkal. The materials for the study were collected from 12 female apparently healthy birds procured from well organized commercial farms in the neighborhood of Namakkal and Erode districts of Tamilnadu. The study was carried out on six mature female birds at their non-laying stage and six female birds at their laying stage for recording the histological and physiological changes in these birds.

The tissues collected from various compartments in oviducts of emu birds were fixed in 10 per cent neutral buffered formalin, Bouin's fluid and Zenker's fluid. Fixed tissues were processed through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax at 58-60 ^{o}C. Paraffin sections of 3-5 µm thickness were cut and utilized for this study. Freshly collected unfixed frozen tissues and post fixed in formal calcium were cut at 20 µm thickness were utilized for demonstration of lipids and enzymes. Routine and special histological techniques *viz.* Ehrlich's Haematoxylin – Eosin method, Van Gieson technique for collagen fibres, Verhoeff's method for elastic fibres, Gomori's method for reticular fibres, PTAH method for muscle fibres, Masson's trichrome technique for connective tissues fibres, Periodic acid-Schiff (PAS) technique for carbohydrates, Combined AB-PAS for Neutral mucopolysaccharides, Millon's reaction for tyrosine, Oil red 'O method for lipids, Best Carmine for glycogen, Gomori's lead method for Acid phosphatase and Gomori's cobalt method for Alkaline phosphatase (Bancroft and Stevens, 1996) were employed on all the sections of oviduct for microanatomical observations,

Micrometrical parameters were recorded using the Leica image processing system and software to record the length and width of mucosal folds, thickness of epithelium and muscular coat of various compartments of oviduct in laying and non laying emu birds.

RESULTS AND DISCUSSIONS

General Histology of Oviduct

The oviduct of the adult emu birds consisted of Tunica mucosa, Tunica muscularis and Tunica serosa from within outwards. The Tunica mucosa showed an epithelium and lamina propria. The mucosa showed longitudinal folds thrown into primary and secondary branches varying in length at different segments of the oviduct. The secondary folds were seen in infundibulum, uterus but less common in the isthmus, vagina and absent in magnum. The mucosa was lined by pseudostratified ciliated columnar epithelium with numerous goblet cells except in funnel shaped part of infundibulum.

The lamina propria was formed by collagen, reticular and elastic fibres and scattered blood capillaries. The lymphocytes, plasma cells and mast cells were noticed in various compartments of the oviduct of adult emu birds. Simple, branched, convoluted tubular glands were observed in the lamina propria of most of the regions of the oviduct. These glands were lined by cuboidal cells and the nuclei were restricted to their basal regions. The length and width of mucosal folds, epithelial thickness and the muscular coat thickness of various segments of oviduct in laying and non-laying emu birds are shown in Table 1.

The height of the epithelium was maximum in vagina and minimum in funnel shaped part of infundibulum.

The tunica muscularis consisted of a thin outer longitudinal and thick inner circular layer of smooth muscle fibres. The thickness of the tunica muscularis was maximum in the vagina which decreased gradually towards the funnel. The tunica serosa was lined by single layer of mesothelial cells and connective tissue fibres.

REGIONAL HISTOLOGY OF OVIDUCT

- **Infundibulum**

The funnel shaped part of the infundibulum was devoid of mucosal folds. In the tubular part the folds were relatively well developed and showed secondary and tertiary folds. The occurrence of primary, secondary and tertiary folds in the mucosa was also reported in ostrich (Saber *et al.*, 2009) and turkey (Parto *et al.*, 2011). The wall of the infundibulum was thin at funnel shaped part and gradually increased in thickness at tubular part in both laying and non-laying emu birds.

The funnel part was lined by simple tall columnar ciliated epithelium (Figure 1) without goblet cells. Evencioneto *et al.* (1997) also noticed cylindrical ciliated epithelium in the funnel part of infundibulum. In contrast, Mohammadpour and Keshtmandi (2008) stated the epithelium as simple cuboidal in turkey and Saber *et al.* (2009) described it as pseudo stratified ciliated columnar epithelium in the upper end of infundibulum in ostrich. The glands were absent in the funnel. The tubular part in emu birds was lined by pseudostratified ciliated columnar epithelium with goblet cells scattered between the secretory columnar cells. Mohammadpour and Keshtmandi (2008) stated that the epithelium of infundibulum was ciliated simple columnar in middle and lower end of infundibulum in turkey.

The glands of the lamina propria were observed throughout the mucosal folds and were opening directly between mucosal folds and glandular crypts. These glands were lined by simple cuboidal epithelial cells (Figure 2). In contrast, Sharaf *et al.* (2012) stated that the tubular glands were lined with pyramidal or tall columnar cells in the infundibulum of ostrich.

- **Magnum**

The primary and secondary mucosal folds (Figure 3) were well developed with epithelial invaginations in to the lamina propria of magnum in laying emu bird. In contrary to these findings, Yoshimura and Ogawa, (1998) stated that the secondary mucosal folds composed of many large duct-like structures in the lamina propria in the guinea fowl.

The tunica mucosa was lined by pseudostratified ciliated columnar epithelium with numerous goblet cells. The core of the mucosal folds was formed of lamina propria. Similar findings were reported in ostrich (Saber *et al.*, 2009). The thickness of the mucosa of the magnum was greater than that of the infundibular neck due to the massive proprial glands.

The tubular glands were more in number and closely packed together in all mucosal folds of the magnum in laying emu birds. However, only few glands in the tip of the mucosal folds were noticed in non-laying emu birds. The glandular cells were comparatively pale and possessed cytoplasmic vacuoles in the non-laying birds.

- **Isthmus**

The primary mucosal folds in the isthmus were long and narrow (Figure 4) compared to those of the magnum. The secondary folds were also seen branching from the primary mucosal folds at various heights. The tall mucosal folds exhibited numerous infoldings in the epithelium as in magnum. The tunica mucosa of the isthmus was lined by pseudostratified ciliated columnar epithelium. In contrast to the present findings, Parto *et al.* (2011) reported that the

epithelium was simple columnar with ciliated and goblet cells in laying turkeys. The glands were numerous at the junctions between folds, whereas they were less densely arranged and resembled the glands of the magnum.

The cells of the proprial glands were cuboidal with basal nucleus. The coarse secretory granules were seen in all the glands located in the lamina propria of mucosal folds. Fitzerald (1969) reported that in the Japanese quail, the secretory granules in the cells of the glands were smaller than those found in the magnal glands and in the case of the hen, the reversal was observed (Gopinath, 1974).

- **Shell Gland (Uterus)**

The wall of the shell gland was thicker than that of the magnum and the isthmus. The mucosal folds in the shell gland were numerous numbering more than 50. They were tall, narrow, compact and showed the secondary folds with epithelial invaginations (Figure 5). The epithelium was pseudostratified with intermittent ciliated columnar cells as also described by Parto *et al.* (2011) in turkey. The current finding is not in direct corroboration with the reports of Mohammadapour (2007) who noticed single layer of short columnar cells over the whole length of the oviduct.

The tubular glands were concentrated more in the lateral and free ends of folds. In contrast, the distribution of tubular glands at the bottom of mucosal folds was denser than that at the apical region. The lamina propria showed more number of capillaries dispersed around the glands. The densely packed epithelium was lined by cuboidal cells with basal nucleus.

The shell gland region was highly vascular with large arteries and veins observed between the muscle layers. A secondary plexus of smaller arteries and veins was observed in the lamina propria at the base of the mucosal folds. Arterioles, venules and lymphatics traversed the connective tissue core of the mucosal folds. Capillaries were observed between the glands and also beneath the mucosal epithelium.

- **Vagina**

A series of broad tall mucosal folds with primary and secondary branches were observed. The number of folds were about 30-35. The mucosa of the vagina was composed of pseudostratified ciliated columnar epithelium. The lining of the vaginal folds was a combination of ciliated, non ciliated, vesicular cells. The lamina propria of the vagina was composed of fibroblast, lymphocytes, neutrophils, mast cells, blood vessels, collagen fibres and lymphatics. Unlike other compartments the vagina was devoid of tubular glands. Isolated neurons were observed in the lamina propria.

The occurrence of sperm host glands in the lamina propria of vagina were described earlier by Ferdous *et al.* (2011) in chicken. The corresponding glands were not encountered in the vagina of emu birds. The tunica muscularis showed distinct inner circular and outer longitudinal layer separated by connective tissue fibres. The tunica muscularis was particularly well developed than in any other part of the oviduct as reported by Gopinath (1974).

HISTOCHEMISTRY OF OVIDUCT

Carbohydrates

The goblet cells in the epithelium of mucosal folds reacted strongly for PAS staining (Figure 6). These cells in the pars minor uteri contained more PAS positive substance than those of the pars major uteri. The secretory substance of the goblet cells shoed intense reaction for acid mucopolysaccharides throughout the oviduct except the isthmus which showed only a weak reaction. Moderate reaction for PAS and weak reaction for acid mucopolysaccharides was observed in the

ciliated cells lining the funnel shaped part of the infundibulum. The apical cytoplasm of the lining epithelium and lamina propria showed strong PAS positive reaction in the mucosal folds of oviduct in emu. The tubular glands showed moderate reaction for PAS and negative reaction for acid mucopolysaccharides throughout the oviduct.

Proteins

Rao (1994) noticed the presence of proteins in the glands of the infundibulum, magnum, isthmus, and the shell gland-vaginal junction in domestic duck. In the current study intense Millon's reaction was observed in the cytoplasm of the oocyte and glands of the magnum. The reaction was moderate in cells lining the grooves between the mucosal folds in the infundibulum, glands of the infundibulum, isthmus, magnum and shell gland. The importance of existence of the disulphide group of proteins could be attributed to their active role in the activate transport of sugars and amino acids across the cell membrane as pointed by Lehninger (1975).

Lipids

The epithelium of the oviduct from shell gland to vagina showed a moderate staining for the lipids. The increased amount of lipids found in epithelium of oviduct of non-laying birds is probably because of their lowered metabolic activity as in toad (Hara and Yamada, 1963) or it might be the age related change.

Enzymes

The alkaline phosphatase activity in the epithelium of uterus and vagina of laying birds was strong. Gulati and Nangia (1974) found a positive correlation between the alkaline phosphatase and the secretory activity in oviduct of chicken.

CONCLUSIONS

From the current study the oviduct of the adult emu birds consisted of Tunica mucosa, Tunica muscularis and Tunica serosa from within outwards. The mucosa showed longitudinal folds thrown into primary and secondary branches varying in length at different segments of the oviduct. The mucosa was lined by pseudostratified ciliated columnar epithelium with numerous goblet cells except in funnel shaped part of infundibulum. The lamina propria was formed by connective tissue fibers and cells. The height of the epithelium was maximum in vagina and minimum in funnel shaped part of infundibulum.

Table 1: Micrometry of Oviduct in Laying and Non-Laying Emu Birds

CATEGORY	SEGMENTS OF OVIDUCT	MUCOSAL FOLD LENGTH (µM)	MUCOSAL FOLD WIDTH (µM)	EPITHELIAL THICKNESS (µM)	MUSCULAR COAT THICKNESS (µM)
LAYING	Infundibulum	831 ± 44.04	458 ±18.70	49.0 ± 1.50	304 ± 4.01
	Magnum	949 ± 29.02	321 ± 16.30	54.6 ± 2.30	447 ± 12.95
	Isthmus	1244 ± 25.30	335 ± 8.40	52.0 ± 1.30	690 ± 38.93
	Uterus	2755 ± 45.12	1140 ± 46.8	60.8 ± 1.0	991 ± 20.47
	Vagina	2164 ± 33.04	868 ± 25.52	99.1 ± 0.90	1072 ± 72.60
NON-LAYING	Infundibulum	273 ± 20.52	234 ± 24.20	19.0 ± 0.30	181 ± 4.50
	Magnum	782 ± 18.02	318 ± 9.87	22.9 ± 0.60	383 ± 4.01
	Isthmus	1056 ± 23.95	267 ± 17.42	22.3 ± 0.15	487 ± 5.21
	Uterus	1372 ± 14.21	380 ± 7.52	30.0 ± 0.80	865 ± 69.30
	Vagina	1174 ± 31.57	303 ± 5.67	17.1 ± 0.30	940 ± 10.21

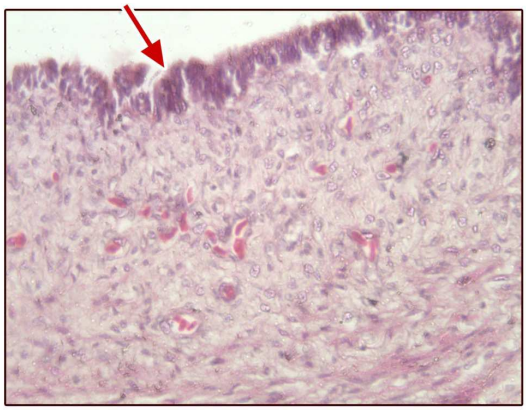

Figure 1: Photomicrograph Showing Funnel Shaped Part of the Infundibulum With Epithelium (Arrow) Without Mucosal Folds. H & E X 100

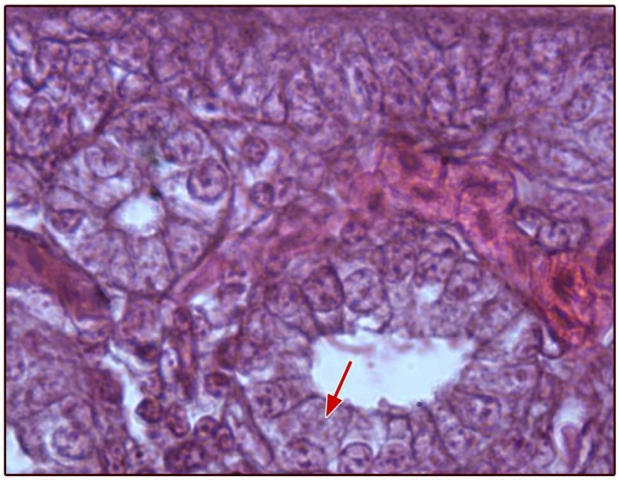

Figure 2: Photomicrograph Showing Coarse Secretory Granules (Arrow) in the Glands of Infundibulum. H & E X 400

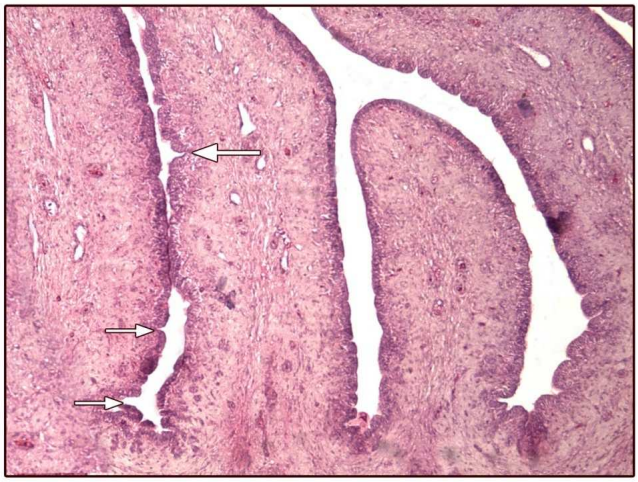

Figure 3: Photomicrograph Showing Secondary Mucosal Folds with Epithelial Invagination (Arrow) into the Lamina Propria of a Laying Bird in Magnum. H & E X 100

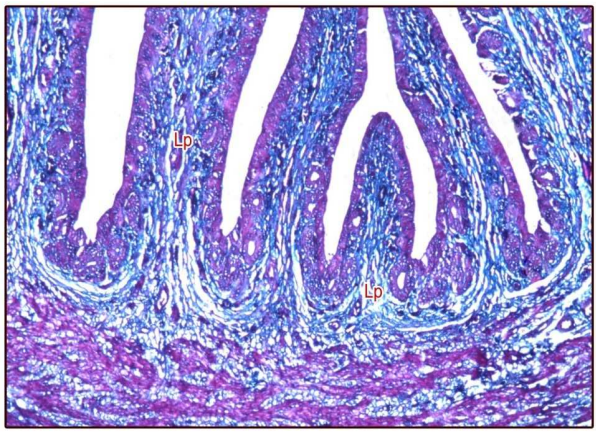

Figure 4: Photomicrograph Showing Long and Narrow Mucosal Folds in Isthmus. Lp – Lamina Propria. Masson's Trichrome X 100

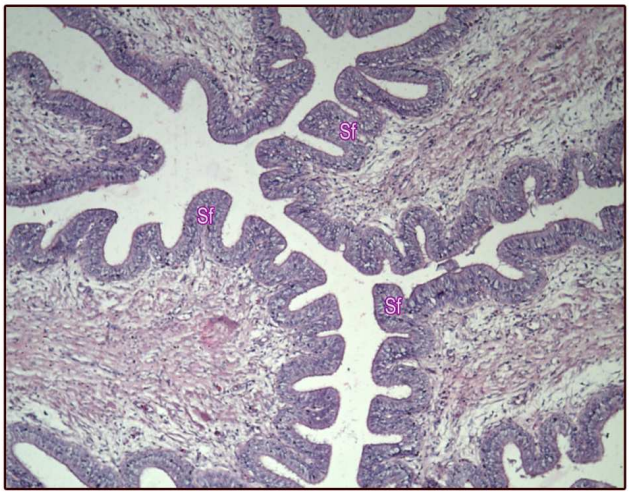

Figure 5: Photomicrograph Showing Secondary Mucosal Folds (Sf) in the Shell Gland. H & E X 100

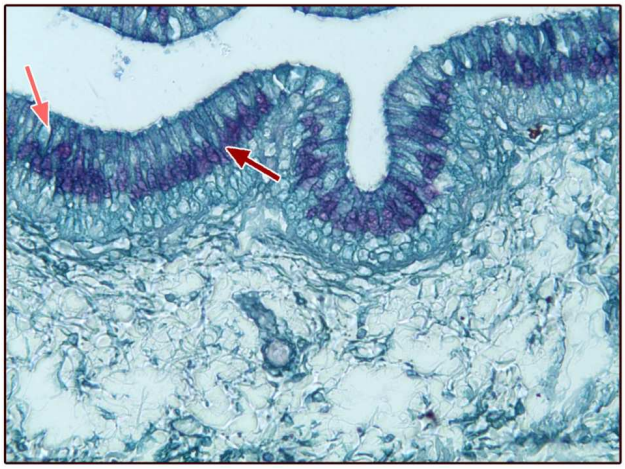

Figure 6: Photomicrograph Showing Strong Reaction in the Goblet Cells (Thin Arrow) and Basement Membrane (Thick Arrow) Of Oviduct for Neutral and Acid Mucopolysaccharides. Alcian Blue – PAS X 400

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